Therapeutic correction of thrombin generation in dilution-induced coagulopathy: Computational analysis based on a data set of healthy subjects

Alexander Y. Mitrophanov, PhD, Frits R. Rosendaal, MD, *and* **Jaques Reifman, PhD,** *Fort Detrick, Maryland*

BACKGROUND: Prothrombin complex concentrates (PCCs), which contain different coagulation proteins, are attractive alternatives to the standard methods to treat dilution-induced (and, generally, traumatic) coagulopathy. We investigated the ability of a novel PCC composition to restore normal thrombin generation in diluted blood. The performance of the proposed PCC composition (coagulation factors [F] II, IX, and X and the anticoagulant antithrombin), designated PCC-AT, was compared with that of FVIIa and PCC-FVII, which is the PCC composition containing FII, FVII, FIX, and FX (main components of most PCCs).

METHODS: We used a thoroughly validated computational model to simulate thrombin generation in normal and diluted blood for 472 healthy subjects in the control group of the Leiden Thrombophilia Study. For every simulated thrombin curve, we calculated and analyzed five standard thrombin generation parameters.

RESULTS: The three therapeutic agents (FVIIa, PCC-FVII, and PCC-AT) caused statistically significant changes in each of the five thrombin generation parameters in diluted blood. Factor VIIa tended to primarily impact clotting time, thrombin peak time, and maximum slope of the thrombin curve, whereas in the case of PCC-FVII, thrombin peak height and the area under the thrombin curve were affected particularly strongly. As a result, these two therapeutics tended to push those respective parameters outside their normal ranges. PCC-AT significantly outperformed both FVIIa and PCC-FVII in its ability to normalize individual thrombin generation parameters in diluted blood. Furthermore, PCC-AT could simultaneously restore all five thrombin generation parameters to their normal levels in every subject in the study group.

CONCLUSIONS: Our computational results suggest that PCC-AT may demonstrate a superior ability to restore normal thrombin generation compared with FVIIa and PCC-FVII. (*J Trauma Acute Care Surg*. 2012;73: S95–S102. Copyright © 2012 by Lippincott Williams & Wilkins)

KEY WORDS: Prothrombin complex concentrates; coagulation factors; factor VIIa; thrombin; antithrombin.

Pathologic conditions, such as trauma, and medical procedures, such as surgery, can lead to the onset of dilution-induced and consumption coagulopathy, which are manifested through a decrease in plasma concentrations of the proteins constituting the biochemical blood coagulation network.[1,2] Current approaches to treat coagulopathy are typically aimed at increasing the levels of functional coagulation proteins in the bloodstream.[3] Combinations of purified clotting factors, termed *prothrombin complex concentrates* (PCCs), may prove efficacious in the treatment of traumatic and surgical bleeding.[4] They could be attractive alternatives to both the frequently used approach based on the use of fresh-frozen plasma (FFP) and a recently emerged approach involving the use of recombinant activated factor VII (rFVIIa), a high-profile therapeutic whose properties need further investigation.[5–7] Yet, the possibility of thromboembolic complications induced by PCC use[8,9] warrants further studies of their effects on hemostasis. A question that arises naturally is that of optimal PCC composition: what combination of clotting factors is necessary and sufficient to guarantee nearly normal blood coagulation, even when the concentrations of other clotting factors are substantially decreased? Here, we propose and analyze a PCC composition that, when added to diluted blood, may accurately restore the thrombin generation curve to its shape observed for normal (i.e., undiluted) blood.

The generation of thrombin, a central component of the biochemical blood coagulation network,[10] is triggered when blood from a damaged vessel comes into contact with tissue factor (TF), a protein expressed on the surface of TF-bearing cells that are exposed to blood at the site of vascular injury.[11] When studied in vitro, thrombin generation is characterized by a peak-shaped thrombin curve, which is typically described by five quantitative parameters: clotting time (CT), thrombin peak time (PT), maximum slope of the thrombin curve (MS), thrombin peak height (PH), and area under the thrombin curve (AUC; Fig. 1). The first three parameters may be regarded as

From the DoD Biotechnology High-Performance Computing Software Applications Institute (A.Y.M., J.R.), Telemedicine and Advanced Technology Research Center, US Army Medical Research and Materiel Command, Fort Detrick, Maryland; and Department of Clinical Epidemiology and Hemostasis and Thrombosis Research Center (F.R.R.), Leiden University Medical Center, Leiden, the Netherlands.

This work was presented at the Advanced Technology Applications for Combat Casualty Care meeting, August 15–18, 2011, Fort Lauderdale, Florida.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.jtrauma.com).

Address for reprints: Alexander Y. Mitrophanov, PhD, or Jaques Reifman, PhD, DoD Biotechnology High-Performance Computing Software Applications Institute, Telemedicine and Advanced Technology Research Center, US Army Medical Research and Materiel Command, ATTN: MCMR-TT, 504 Scott St, Fort Detrick, MD 21702; emails: alex@bhsai.org (A.Y.M.); jaques.reifman@us.army.mil (J.R.).

DOI: 10.1097/TA.0b013e3182609bca

Copyright © 2012 Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Report Documentation Page

Form Approved
OMB No. 0704-0188

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
2012		**00-00-2012 to 00-00-2012**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Therapeutic correction of thrombin generation in dilution-induced coagulopathy: Computational analysis based on a data set of healthy subjects	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **U.S. Army Medical Research and Materiel Command,Biotechnology High Performance Computing Software Applications Institute,Telemedicine and Advanced Technology Research Center,Fort Detrick,MD,21702**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**8**	

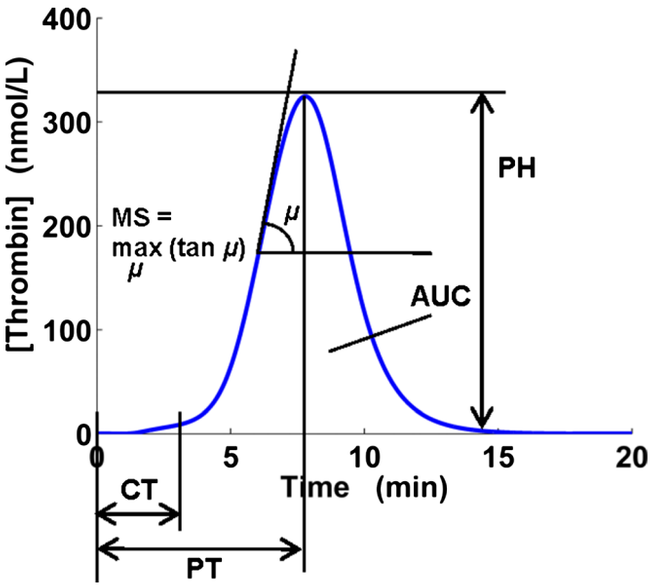

Figure 1. Quantitative parameters of thrombin generation: the timing parameters (CT [time to 10 nmol/L thrombin], PT, and MS) and the amount parameters (PH and AUC). The thrombin curve was computationally generated for normal blood composition.

the timing parameters, whereas the last two parameters are the amount parameters.[7] The ability of a hemostatic agent to restore thrombin generation can thus be assessed by its ability to restore normal values of these five parameters.

The main constituents of existing PCCs are the four coagulation factors (F) II (prothrombin), FVII, FIX, and FX.[3] Notably, FVII inhibits thrombin generation by competing with its own activated form, FVIIa, for binding with TF.[12] The reported possibility of thromboembolic complications during PCC use[8,9] implies that the action of standard PCCs may be intrinsically unbalanced because of the dominant procoagulant effects of FII, FIX, and FX. Experimental evidence indicates that antithrombin (AT), which deactivates thrombin by direct binding, may be the most potent of the natural anticoagulant mechanisms.[13,14] We thus hypothesized that replacing FVII with AT might result in a more balanced PCC action and, therefore, a more accurate restoration of normal thrombin generation in human subjects. We tested this hypothesis by performing a computational analysis in which we used a computational kinetic model of in vitro thrombin accumulation to generate and analyze thrombin curves for the 472 control subjects in the Leiden Thrombophilia Study (LETS).[15] The computational model has been thoroughly validated and demonstrated reasonable accuracy when benchmarked against experimental data.[7,16,17] For each subject, we compared and contrasted the action of three therapeutic agents: FVIIa, PCC-FVII (a combination of FII, FVII, FIX, and FX), and PCC-AT (a combination of FII, FIX, FX, and AT).

METHODS

Mathematical Modeling

We performed a computational analysis of an experimental data set collected in the course of the previously published LETS[15] (thus, no institutional review board approval/patient consent was needed). LETS is a large-scale case-control study focused on assessing the importance of the various thrombosis risk factors. Our data set consisted of coagulation factor measurements for 472 healthy subjects constituting the LETS control group. (Of the 474 healthy control subjects LETS, coagulation factor measurements were available for 472 individuals, because one of the subjects was receiving warfarin and was excluded, and for another subject, no TF pathway inhibitor-level measurements were available.) For each subject, we used the updated version[18] of the kinetic model developed in Kenneth Mann's laboratory[13] to generate and analyze thrombin curves in *normal* (i.e., undiluted) blood, *diluted* (i.e., pretreatment) blood, and *posttreatment* blood (i.e., diluted blood supplemented with therapeutic agents). We also performed an analysis for the "average" subject, that is, a subject whose normal coagulation factor levels are equal to the corresponding average values in human plasma.[13] The model simulates thrombin generation in plasma under the assumption that platelets are fully activated. The model was implemented in the SimBiology toolbox of the MATLAB software suite (MathWorks, Natick, MA) as described in Mitrophanov and Reifman;[7] all computations were performed in MATLAB 2010b. The generation and analysis of thrombin curves were carried out as described in Mitrophanov and Reifman.[7]

Blood dilution in the model was intended to reflect the dilution of coagulation factors driven primarily by resuscitation fluid infusion. Existing evidence suggests that the most realistic representation of blood dilution in vivo is an unequal dilution model, where the degrees of dilution for different coagulation proteins are different.[5,19,20] Unequal dilution may be attributed to complex interactions of different coagulation factors with the vasculature during dilution.[21] We thus used an unequal dilution model based on an in vivo porcine experimental study that resulted in induced coagulopathy.[19] In our model, blood dilution was effected by multiplying the initial concentrations of clotting factors by the dilution factors shown in Supplemental Digital Content 1 (http://links.lww.com/TA/A131). Simulated supplementation of diluted blood with FVIIa in the model was effected by increasing the initial FVIIa concentration by 40 nmol/L (which approximately corresponds to 90 μg/kg body weight, a standard therapeutic dose for rFVIIa) or by a smaller amount. Simulated supplementation of diluted blood with PCC-FVII and PCC-AT in each subject was effected by setting the initial concentrations of the corresponding PCC components to their normal (*final*) values in that subject. Our computational model does not contain the necessary modules to map PCC component concentrations *before* PCC is added to diluted blood to their final concentrations *after* PCC addition. However, existing evidence demonstrates that it is possible to choose presupplementation PCC component concentrations that would give the desired PCC component normalization in vivo.[5,6] The concentration of TF (which initiates thrombin generation in the model) was not affected by dilution and was equal to 5 pmol/L, a standard

© 2012 Lippincott Williams & Wilkins

Copyright © 2012 Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

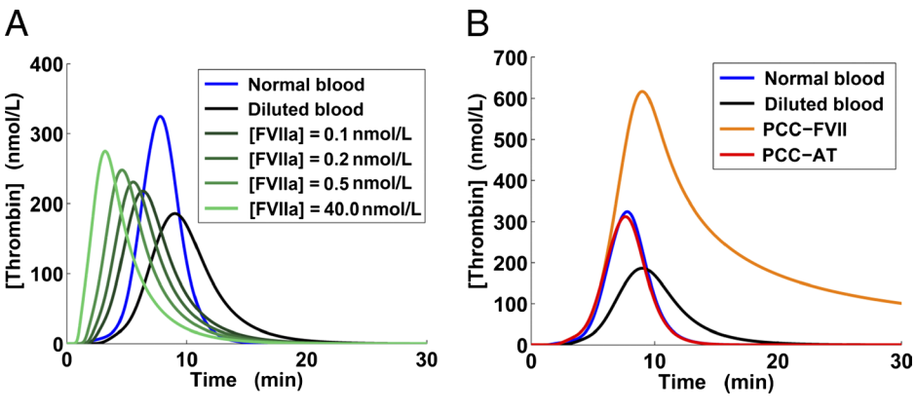

Figure 2. Action of the therapeutics on diluted blood in the ''average'' subject. ''Diluted'' blood designates pretreatment blood. *A*, Diluted blood was supplemented with coagulation factor (F)VIIa at various concentrations (*green curves*). The incremental character of the curves corresponding to nonincremental FVIIa doses seems to result from the FVIIa saturation effect. *B*, Diluted blood was supplemented with PCC-FVII or PCC-AT (see Methods for the supplementation scenario).

value used in in vitro studies.[7,16] Thrombin curves were generated during a 40-minute time interval.

Statistical Analysis

Differences between distributions were tested using the two-sided Wilcoxon signed rank test. The propensity of various effects at the subject level was characterized quantitatively by estimating their probabilities, that is, calculating the fractions (or percentages) of subjects for whom the effect was detected. The standard error (SE) in the estimation of the probabilities was calculated as follows: $SE = \sqrt{q(1-q)/N}$, where q is the probability estimate and $N = 472$ is the subject group size.[22] Note that SE = 0 when q equals 0 or 1; when this was the case, the SE was omitted in the text.

RESULTS

Because our results were obtained computationally, blood dilution and therapeutic supplementation described below correspond to simulated results. No actual blood products were used.

Correction of Thrombin Generation in the ''Average'' Subject

To perform an initial comparison of the therapeutic agents considered in this study, we calculated their effects on thrombin generation in the "average" subject. Diluted blood in the "average" subject was supplemented with FVIIa at different concentrations ranging from 0.10 nmol/L (average concentration in normal blood plasma) to 40 nmol/L (Fig. 2*A*). Although FVIIa could improve thrombin generation parameters affected by dilution, its action did not produce a thrombin curve that would be close to the thrombin curve in normal blood. Indeed, when 0.10 nmol/L FVIIa was added, PH was only 67.04% of the normal value, but PT was already smaller than its value in normal blood. Further increases in the FVIIa concentration resulted in further improvements in PH (which, even for 40-nmol/L added FVIIa, was still lower than its normal value), but the timing of thrombin generation was considerably accelerated compared with normal blood (Fig. 2*A*).

Supplementation of diluted blood with PCC-FVII led to a rather accurate restoration of some of the timing parameters of the thrombin generation curve (Fig. 2*B*). Indeed, the differences between CT and PT for normal blood and posttreatment blood were 13.28% and 15.68%, respectively. For MS,

TABLE 1. Thrombin Generation Parameters in the Subject Group

Blood Type	CT, min	PT, min	MS, nmol/L/min	PH, nmol/L	AUC, nmol/L × min
Normal blood	3.31 (2.99–3.73)	7.90 (7.43–8.49)	121.28 (98.89–145.28)	320.34 (276.06–368.27)	1,304.27 (1,153.38–1,481.32)
Diluted blood	3.95 (3.65–4.26)	9.15 (8.66–9.72)	54.56 (44.81–66.16)	185.53 (160.56–208.69)	1,160.50 (1,029.39–1,315.69)
Diluted blood with 40 nmol/L FVIIa	0.95 (0.90–1.01)	3.25 (3.10–3.39)	151.01 (129.30–175.92)	273.06 (242.63–300.88)	1,142.89 (1,012.10–1,298.27)
Diluted blood with PCC-FVII	2.89 (2.70–3.08)	9.14 (8.69–9.64)	183.12 (151.07–213.82)	614.83 (532.85–690.49)	7,130.42 (5,264.43–9,866.00)
Diluted blood with PCC-AT	2.88 (2.64–3.14)	7.79 (7.31–8.26)	108.91 (88.03–134.12)	307.89 (264.81–353.89)	1,306.15 (1,155.66–1,483.62)

Values are medians (interquartile ranges).

Statistical significance of differences (each thrombin generation parameter tested independently): diluted (i.e., pretreatment) blood versus normal blood, $p < 1.0 \times 10^{-76}$; diluted blood versus posttreatment blood, $p = 0.01$ for PT when diluted blood was supplemented with PCC-FVII, and $p < 1.0 \times 10^{-78}$ in all other cases.

Copyright © 2012 Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

this difference was 49.18%. Both PH and AUC in posttreatment blood substantially exceeded their values in normal blood (Fig. 2*B*). Taken together, these results indicate that the overall action of PCC-FVII biases thrombin generation toward a prothrombotic state. In contrast, the addition of PCC-AT to diluted blood resulted in an accurate restoration of the full thrombin generation curve (Fig. 2*B*).

Therapeutic Modulation of the Thrombin Generation Parameters in the Subject Group

The medians and interquartile ranges for the numerically simulated thrombin generation parameters for normal, diluted, and posttreatment blood in the subject group are shown in Table 1. We focused on the comparisons between normal and diluted-blood (i.e., pretreatment) groups and between diluted-blood

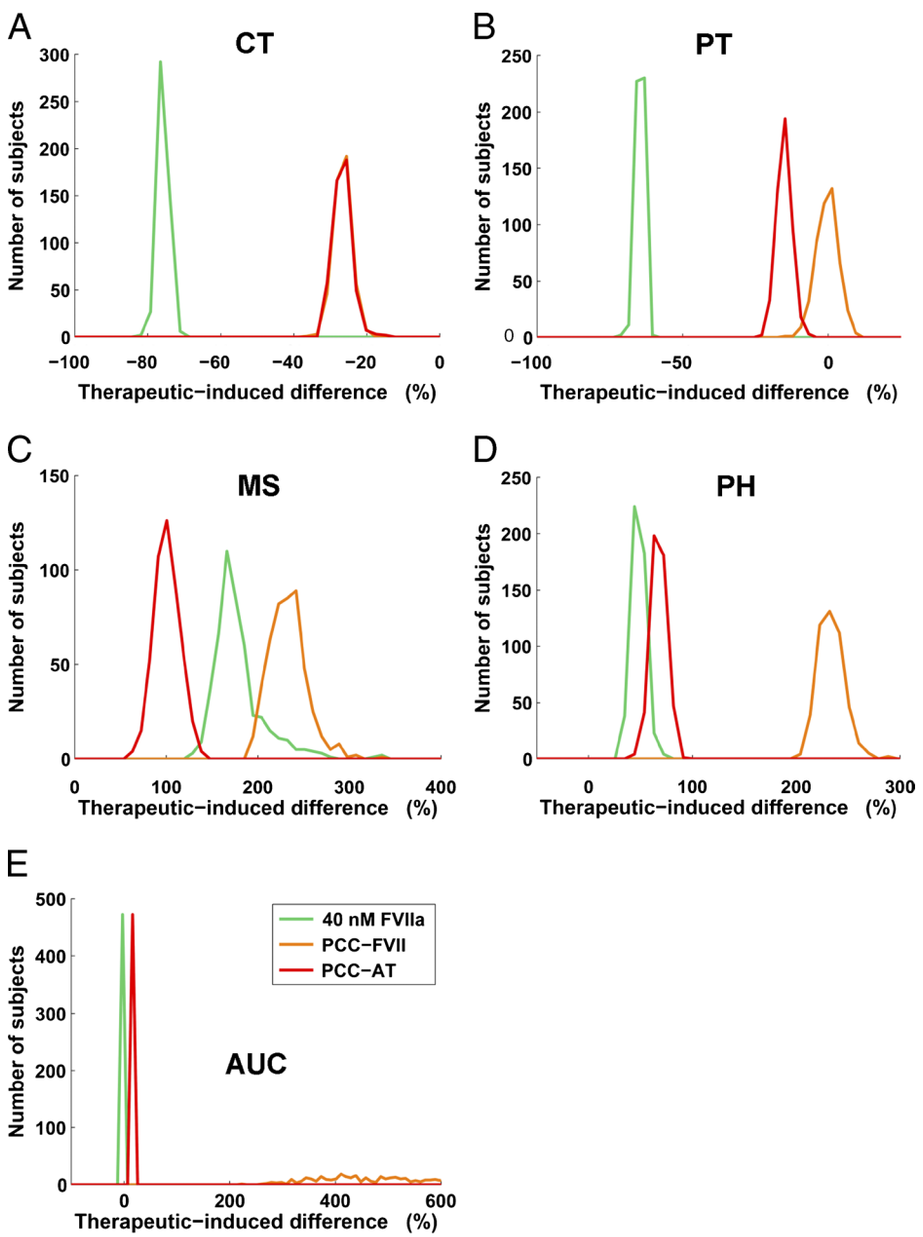

Figure 3. Distributions of therapeutic-induced differences for thrombin generation parameters in the study group. For each subject, the therapeutic-induced difference = [(parameter value in posttreatment blood) − (parameter value in diluted blood)] / (parameter value in diluted blood) × 100%, where ''diluted'' blood designates pretreatment blood. Plots were generated using the MATLAB function HIST with 150 bins. The curves represent all 472 subjects in our study group: CT (*A*), PT (*B*), MS (*C*), PH (*D*), and AUC (*E*). In the subplot for CT, the distribution for PCC-FVII almost coincided with that for PCC-AT.

© 2012 Lippincott Williams & Wilkins

Copyright © 2012 Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

and posttreatment groups. Our results for the "average" subject (Fig. 2) suggested that the ability of FVIIa to decrease CT and PT (i.e., to accelerate thrombin generation) in diluted blood generally exceeds that of PCC-FVII and PCC-AT. Indeed, this was confirmed for all subjects in our subject group (Fig. 3, *A* and *B*). As a result of adding PCC-AT to diluted blood, PT decreased in all subjects, whereas PCC-FVII caused a decrease in PT in 52.12% (SE, 2.30%) of the subjects and an increase in PT in the rest of the subjects (Fig. 3*B*). All three therapeutic strategies increased MS and PH in all subjects, with PCC-FVII inducing large changes in higher numbers of subjects compared with FVIIa and PCC-AT (Fig. 3, *C* and *D*). In all of the subjects, the increase in PH induced by PCC-FVII exceeded 200% (with respect to the values in diluted blood; Fig. 3*D*). Likewise, in 96.82% (SE, 0.81%) of the subjects, the increase in AUC induced by PCC-FVII exceeded 300% (Fig. 3*E*). These results are in agreement with the significant increases in PH and AUC induced by PCC-FVII in the "average" subject (Fig. 2*B*). The AUC was practically unaffected by FVIIa and PCC-AT (Fig. 3*E*).

Correction of Thrombin Generation in the Subject Group

The median values and interquartile ranges for the absolute values of simulated normalization errors (i.e., the differences between the thrombin generation parameters in posttreatment blood and the corresponding values in normal blood) in the subject group are shown in Table 2. Here we focused on the comparisons of posttreatment groups for different therapeutics. For all thrombin parameters, the absolute normalization errors were significantly smaller for PCC-AT than for FVIIa. When compared with PCC-FVII, PCC-AT yielded significantly smaller absolute normalization errors for PT, MS, PH, and AUC. For CT, the very similar medians and interquartile ranges suggested no significant differences between the effects of PCC-AT and PCC-FVII.

The absolute value of the CT and PT normalization errors for FVIIa exceeded ~50% in all subjects, whereas both PCC-based strategies induced smaller normalization errors (Fig. 4, *A* and *B*). The magnitude (i.e., the absolute value) of CT normalization errors for PCC-treated and untreated diluted blood were comparable (Fig. 4*A*). All other thrombin generation parameters were considerably closer to their normal values for PCC-AT–supplemented diluted blood than for the other two therapeutic supplements and for supplement-free diluted blood. Indeed, the numbers of subjects with small normalization errors were higher for PCC-AT than for the other two therapeutics (Fig. 4, *B–E*). The comparatively better ability of PCC-AT to restore normal thrombin generation was particularly well pronounced for MS and PH (Fig. 4, *C* and *D*). Supplementation with FVIIa or PCC-AT resulted in AUC values very close to the normal values (Fig. 4*E*). In contrast, supplementation with PCC-FVII resulted in AUC values much larger than normal in most subjects (Fig. 4*E*), which was consistent with our results for the "average" subject (Fig. 2).

The superior ability of PCC-AT to restore normal values of thrombin generation parameters was confirmed by a subject-level analysis. For each subject, we calculated the magnitudes of normalization errors for each of the three therapeutic strategies and each of the five thrombin generation parameters. For CT, these magnitudes were the smallest for PCC-AT-supplemented blood in 51.91% (SE, 2.30%) of the subjects. For PT, MS, PH, and AUC, the corresponding fractions were 98.31% (SE, 0.59%), 82.84% (SE, 1.74%), 93.64% (SE, 1.12%), and 100%, respectively.

Simultaneous Normalization of Thrombin Generation Parameters

Normal thrombin generation in coagulopathy cannot be fully restored unless several thrombin generation parameters are normalized simultaneously in the same subject. We calculated the probabilities of simultaneous normalization for three groups of parameters: the timing parameters, the amount parameters, and all five thrombin generation parameters. In those computations, a thrombin parameter in a subject was considered abnormal if it was more than 1.5-fold different from its normal value in the same subject. This threshold was chosen to make our results compatible and comparable with an operational definition of traumatic coagulopathy, according to which a patient is considered coagulopathic if his or her prothrombin time is 1.5-fold larger than normal.[1]

On supplementation of diluted blood with PCC-AT, all thrombin generation parameters were normalized in all subjects. In contrast, none of the subjects whose diluted blood was supplemented with FVIIa or PCC-FVII had all of their thrombin generation parameters normalized. PCC-FVII was able to normalize all three timing parameters in 49.15% (SE, 2.30%) of the subject group but failed to simultaneously normalize the two amount parameters in any of the subjects. Factor VIIa normalized the amount parameters in all of the subjects and simultaneously normalized three thrombin parameters (MS, PH, and AUC) in

TABLE 2. Absolute Differences Between the Thrombin Generation Parameters in Posttreatment Blood and the Corresponding Values in Normal Blood in the Subject Group

Therapeutic	CT, min	PT, min	MS, nmol/L/min	PH, nmol/L	AUC, nmol/L × min
FVIIa	2.37 (2.09–2.73)	4.65 (4.26–5.09)	29.58 (19.56–40.33)	48.38 (30.03–66.75)	160.71 (139.88–183.61)
PCC-FVII	0.44 (0.27–0.67)	1.23 (1.07–1.37)	58.73 (50.54–69.67)	287.12 (253.39–324.22)	584.11 (410.97–837.58)
PCC-AT	0.44 (0.24–0.63)	0.20 (0.10–0.34)	10.75 (8.26–13.94)	12.11 (9.43–15.65)	2.31 (1.84–2.97)

Values are medians (interquartile ranges).

Statistical significance of differences (each thrombin generation parameter tested independently): PCC-AT versus FVIIa, $p < 1.0 \times 10^{-56}$; PCC-AT versus PCC-FVII, $p = 0$ to machine precision for PT, MS, PH, and AUC, and $p = 0.10$ for CT.

Copyright © 2012 Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

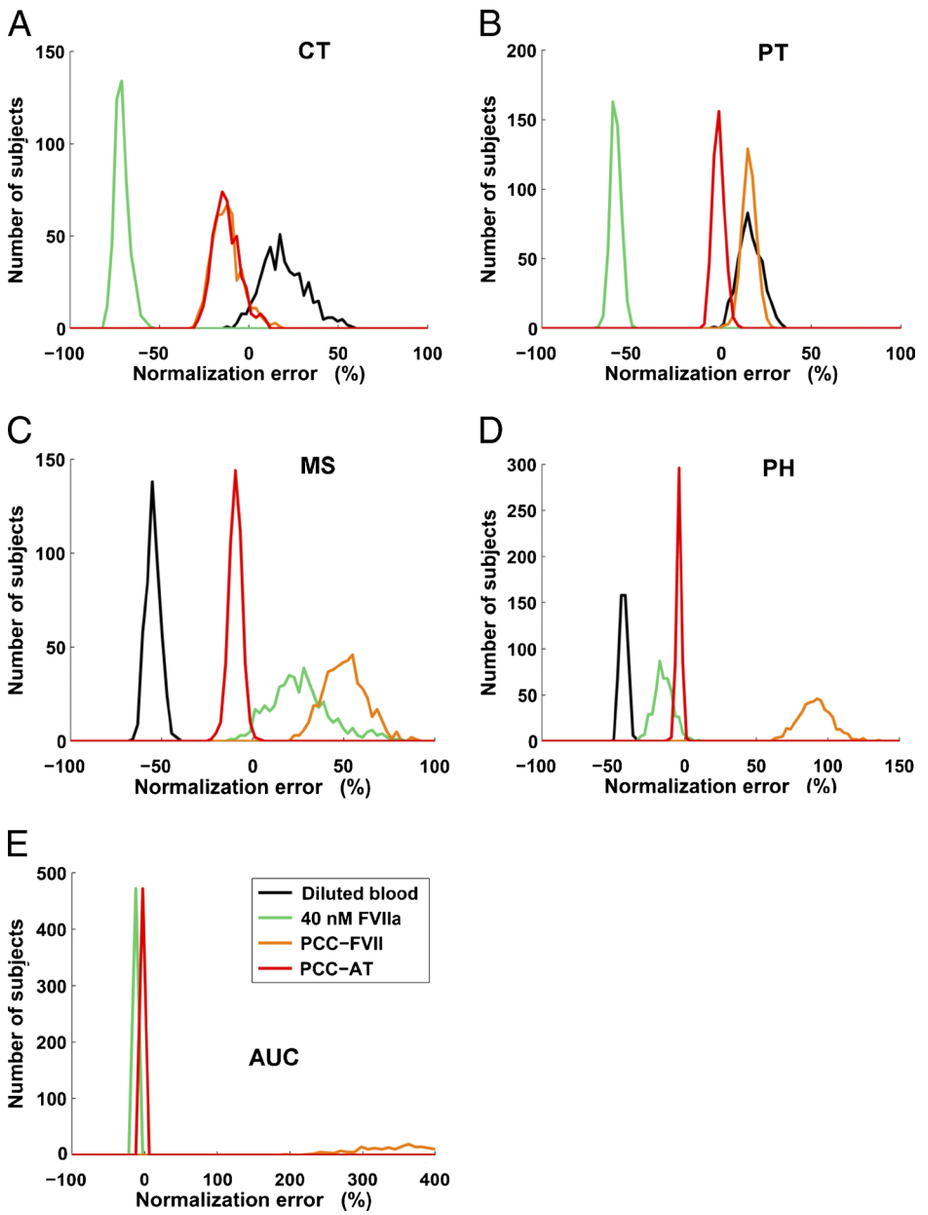

Figure 4. Distributions of normalization errors for thrombin generation parameters in the study group. For each subject, normalization error = [(parameter value in diluted or posttreatment blood) − (parameter value in normal blood)] / (parameter value in normal blood) × 100%, where ''diluted'' blood designates pretreatment blood. Plots were generated using the MATLAB function HIST with 150 bins. The curves represent all 472 subjects in our study group: CT (*A*), PT (*B*), MS (*C*), PH (*D*), and AUC (*E*). The distribution of AUC normalization errors for diluted blood with no therapeutic supplements coincided with that for diluted blood supplemented with FVIIa.

90.47% (SE, 1.35%) of the subjects. Yet, FVIIa could not simultaneously normalize all of the timing parameters in any of the subjects.

DISCUSSION

In our computations, all three considered therapeutics (FVIIa, PCC-FVII, and PCC-AT) could improve thrombin generation in diluted blood by counteracting the effects of dilution (Figs. 2 and 3 and Table 1). However, the high impact of FVIIa on the timing parameters (Fig. 3, *A* and *B*) tended to push them outside the normal ranges while leaving the amount parameters at levels somewhat below normal (Figs. 2*A* and 4 and Table 1). In contrast, the use of PCC-FVII resulted in reasonably accurate normalization of the two timing parameters, CT and PT, whereas the remaining three parameters had values that were noticeably higher than normal (Figs. 2*B* and 4 and Table 1). PCC-AT was similar to PCC-FVII in its

© 2012 Lippincott Williams & Wilkins

Copyright © 2012 Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

ability to normalize CT (Fig. 4*A* and Tables 1 and 2). For all other thrombin generation parameters considered independently, and for groups of parameters considered simultaneously, PCC-AT demonstrated a notably higher ability to normalize the parameters than FVIIa and PCC-FVII (Fig. 4, *B–E*, and Tables 1 and 2). Our computational modeling results suggest a possible approach to testing the hypothesis about PCC-AT performance with real blood samples.

The normal function of the hemostatic system relies on the concerted action of its procoagulant and anticoagulant components to maintain a fine balance between hemorrhage and thrombosis.[23,24] Because this balance is intrinsic to the biochemical thrombin generation network, we propose that the same principle of balanced action should be applied when designing a therapeutic strategy. The computationally predicted ability of PCC-AT to normalize all thrombin generation parameters may be attributed primarily to the balanced action of the constituting procoagulants (FII, FIX, and FX) and the powerful natural anticoagulant (AT). Notably, the four constituents of PCC-AT are the four most abundant (on the average) main proteins involved in thrombin generation in human blood plasma, with AT being the most abundant component.[13] Our results imply that these proteins are the central functional elements of the biochemical thrombin generation network, and having them at normal levels in the bloodstream may be sufficient for nearly normal thrombin generation.

In contrast, both FVIIa and PCC-FVII in our computations acted as strong procoagulants, and their activity skewed thrombin generation profiles. The unbalanced action of FVIIa was elucidated in a recent work of Mitrophanov and Reifman[7] (see also related work in Monroe[25] and Tanaka et al.[26]). They found that, in the "average" subject, FVIIa primarily impacts CT, PT, and MS, whereas PH and AUC are affected much less. This result led the authors to suggest that, for certain pathologic conditions, FVIIa may not be able to restore all five parameters simultaneously and may result in higher-than-normal thrombin generation rates, which was indeed demonstrated in the current study for the case of dilution-induced coagulopathy. Moreover, here we detected a lack of balance for PCC-FVII, which modulated MS, PH, and AUC much more strongly than it did CT and PT, resulting in abnormally high posttreatment values of MS, PH, and AUC. These examples imply that therapeutics with unbalanced action, while providing a certain level of efficacy, may be intrinsically unsafe. It is therefore likely that thromboembolic adverse effects, whose possibility was reported for rFVIIa[27,28] and PCCs with standard composition (based on PCC-FVII),[8,9] may be attributed to their inherently unbalanced action on thrombin generation.

The limitations of this study are related to its computational nature and its focus on thrombin generation. The numerical model used in this study does not account for fibrin(ogen) and thus cannot be used to directly predict if hemostasis will be achieved. Yet, the generation of thrombin, which converts fibrinogen to fibrin, is a strong determinant of actual clot formation.[29] This process is accurately captured by the computational model. However, the model was originally developed to represent in vitro systems that do not contain the elements of the protein C anticoagulant system.[13,18] Thus, our study does not address the effects of proteins C and S, which are present in some PCC formulations.[19] Another potential limitation stems from our use of blood dilution models based on healthy subject data. Indeed, a more accurate analysis would require the use of data obtained directly from trauma patients with dilution-induced coagulopathy. Moreover, coagulopathy in trauma patients might have a more complex nature than dilution-induced coagulopathy and involve such additional factors as hypothermia and acidosis.[2] Further research is warranted to extend our approach to provide a more comprehensive and in vivo–relevant analysis of traumatic coagulopathy and to experimentally test our computational predictions.

The frequently used approach to treat dilution-induced coagulopathy is through the use of FFP.[3,30] However, although all necessary coagulation proteins are present in FFP, they are diluted, cooled to subphysiological temperatures, and may be partially deactivated, which may decrease the therapeutic efficacy of FFP administration; blood-borne pathogens constitute another danger of FFP transfusions.[3] Because of their convenience in use, PCCs that rely on highly purified or recombinant proteins can be an attractive alternative to FFP, if they can provide the necessary level of efficacy and safety. Our computational results indicate that PCCs, whose main components are FII, FIX, FX, and AT, may become promising therapeutics to treat dilution-induced coagulopathy.

AUTHORSHIP

A.Y.M. and J.R. designed this study. A.Y.M. and F.R.R. collected the data, which A.Y.M. and J.R. analyzed and interpreted. A.Y.M. and J.R. prepared the manuscript.

ACKNOWLEDGMENTS

The authors are grateful to two anonymous reviewers for their insightful comments on an earlier version of this article.

DISCLOSURE

A.Y.M. and J.R. were supported by the Military Operational Medicine Research Program of the US Army Medical Research and Materiel Command, Fort Detrick, Maryland, as part of the US Army's Network Science Initiative. The opinions and assertions contained herein are private views of the authors and are not to be construed as official or as reflecting the views of the US Army or the US Department of Defense. This article has been approved for public release with unlimited distribution.

REFERENCES

1. Hardy JF, De Moerloose P, Samama M. Massive transfusion and coagulopathy: pathophysiology and implications for clinical management. *Can J Anesth*. 2004;51:293–310.
2. Hess JR, Brohi K, Dutton RP, Hauser CJ, Holcomb JB, Kluger Y, Mackway-Jones K, Parr MJ, Rizoli SB, Yukioka T, Hoyt DB, Bouillon B. The coagulopathy of trauma: a review of mechanisms. *J Trauma*. 2008;65:748–754.
3. Fries D, Innerhofer P, Schobersberger W. Time for changing coagulation management in trauma-related massive bleeding. *Curr Opin Anesthesiol*. 2009;22:267–274.
4. Schick KS, Fertmann JM, Jauch KW, Hoffmann JN. Prothrombin complex concentrate in surgical patients: retrospective evaluation of vitamin K antagonist reversal and treatment of severe bleeding. *Crit Care*. 2009;13:R191.
5. Dickneite G, Dörr B, Kaspereit F, Tanaka KA. Prothrombin complex concentrate versus recombinant factor VIIa for reversal of hemodilutional coagulopathy in a porcine trauma model. *J Trauma*. 2010;68:1151–1157.
6. Dickneite G, Pragst I. Prothrombin complex concentrate vs fresh frozen plasma for reversal of dilutional coagulopathy in a porcine trauma model. *Br J Anaesth*. 2009;102:345–354.

Copyright © 2012 Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

7. Mitrophanov AY, Reifman J. Kinetic modeling sheds light on the mode of action of recombinant factor VIIa on thrombin generation. *Thromb Res*. 2011;128:381–390.
8. Grottke O, Braunschweig T, Spronk HM, Esch S, Rieg AD, van Oerle R, Ten Cate H, Fitzner C, Tolba R, Rossaint R. Increasing concentrations of prothrombin complex concentrate induce DIC in a pig model of coagulopathy with blunt liver injury. *Blood*. 2011;118:1943–1951.
9. Rossaint R, Bouillon B, Cerny V, Coats TJ, Duranteau J, Fernández-Mondéjar E, Hunt BJ, Komadina R, Nardi G, Neugebauer E, Ozier Y, Riddez L, Schultz A, Stahel PF, Vincent J-L, Spahn DR. Management of bleeding following major trauma: an updated European guideline. *Crit Care*. 2010;14:R52.
10. Crawley JT, Zanardelli S, Chion CK, Lane DA. The central role of thrombin in hemostasis. *J Thromb Haemost*. 2007;5(Suppl 1):95–101.
11. Hoffman M, Monroe DM. Coagulation 2006: a modern view of hemostasis. *Hematol Oncol Clin North Am*. 2007;21:1–11.
12. van't Veer C, Golden NJ, Mann KG. Inhibition of thrombin generation by the zymogen factor VII: implications for the treatment of hemophilia A by factor VIIa. *Blood*. 2000;95:1330–1335.
13. Hockin MF, Jones KJ, Everse SJ, Mann KG. A model for the stoichiometric regulation of blood coagulation. *J Biol Chem*. 2002;277:18322–18333.
14. van 't Veer C, Golden NJ, Kalafatis M, Mann KG. Inhibitory mechanism of the protein C pathway on tissue factor–induced thrombin generation. Synergistic effect in combination with tissue factor pathway inhibitor. *J Biol Chem*. 1997;272:7983–7994.
15. van der Meer FJ, Koster T, Vandenbroucke JP, Briët E, Rosendaal FR. The Leiden Thrombophilia Study (LETS). *Thromb Haemost*. 1997;78: 631–635.
16. Brummel-Ziedins KE, Orfeo T, Rosendaal FR, Undas A, Rivard GE, Butenas S, Mann KG. Empirical and theoretical phenotypic discrimination. *J Thromb Haemost*. 2009;7(suppl 1):181–186.
17. Orfeo T, Butenas S, Brummel-Ziedins KE, Mann KG. The tissue factor requirement in blood coagulation. *J Biol Chem*. 2005;280:42887–42896.
18. Danforth CM, Orfeo T, Mann KG, Brummel-Ziedins KE, Everse SJ. The impact of uncertainty in a blood coagulation model. *Math Med Biol*. 2009;26:323–336.
19. Dickneite G, Doerr B, Kaspereit F. Characterization of the coagulation deficit in porcine dilutional coagulopathy and substitution with a prothrombin complex concentrate. *Anesth Analg*. 2008;106:1070–1077.
20. Kheirabadi BS, Crissey JM, Deguzman R, Holcomb JB. In vivo bleeding time and in vitro thrombelastography measurements are better indicators of dilutional hypothermic coagulopathy than prothrombin time. *J Trauma*. 2007;62:1352–1359; discussion 1359–1361.
21. Spahn DR, Rossaint R. Coagulopathy and blood component transfusion in trauma. *Br J Anaesth*. 2005;95:130–139.
22. Glantz SA. *Primer of Biostatistics*. 6th ed. New York, NY: McGraw-Hill; 2005.
23. Diamond SL. Systems biology to predict blood function. *J Thromb Haemost*. 2009;7(suppl 1):177–180.
24. Mann KG, Butenas S, Brummel K. The dynamics of thrombin formation. *Arterioscler Thromb Vasc Biol*. 2003;23:17–25.
25. Monroe DM. Modeling the action of factor VIIa in dilutional coagulopathy. *Thromb Res*. 2008;122(suppl 1):S7–S10.
26. Tanaka KA, Taketomi T, Szlam F, Calatzis A, Levy JH. Improved clot formation by combined administration of activated factor VII (NovoSeven) and fibrinogen (Haemocomplettan P). *Anesth Analg*. 2008;106:732–738.
27. O'Connell KA, Wood JJ, Wise RP, Lozier JN, Braun MM. Thromboembolic adverse effects after use of recombinant human factor VIIa. *JAMA*. 2006;295:293–298.
28. Thomas GO, Dutton RP, Hemlock B, Stein DM, Hyder M, Shere-Wolfe R, Hess JR, Scalea TM. Thromboembolic complications associated with factor VIIa administration. *J Trauma*. 2007;62:564–569.
29. Hemker HC, Al Dieri R, De Smedt E, Béguin S. Thrombin generation, a function test of the haemostatic-thrombotic system. *Thromb Haemost*. 2006;96:553–561.
30. Shuja F, Shults C, Duggan M, Tabbara M, Butt MU, Fischer TH, Schreiber MA, Tieu B, Holcomb JB, Sondeen JL, et al. Development and testing of freeze-dried plasma for the treatment of trauma-associated coagulopathy. *J Trauma*. 2008;65:975–985.

© 2012 Lippincott Williams & Wilkins

Copyright © 2012 Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.